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Effects of in-vivo administration of a monoclonal antibody specific for the interleukin-2 receptor on the acute graft-versus-host reaction in mice

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SUMMARY

Parental strain T lymphocyte injected into F1 mice respond to allogeneic MHC antigens and so induce the symptoms of a graft-versus-host reaction (GVHR). We have measured the local GVHR by the popliteal lymph node assay, and showed the suppression of the local GVHR in mice by treatment with the monoclonal antibody (MoAb) AMT-13 which is specific against the interleukin 2 (IL-2) receptor on activated mouse lymphocytes. The inhibitory effect of the AMT-13 administration was comparable with the suppression of the local GVHR by treatment with L3T4, an MoAb directed against the T helper subset. The L3T4 administration caused a dramatic decrease in the proportion of the cells with the L3T4 phenotype in the circulation and a marginal reduction of these cells in the lymph nodes. In contrast, the AMT-13 treated mice showed no changes in the distribution of the T lymphocyte subsets besides those in the GVHR-stimulated lymph nodes. Obviously, only the small subset of antigen-activated IL-2 receptor-bearing lymphocytes was influenced by treatment with AMT-13. MoAb directed against antigens whose expression is restricted to activated lymphocytes, such as the IL-2 receptor, might become useful for a short term immunosuppression with limited side effects.

Keywords IL-2 receptor-bearing cells graft-versus-host reaction

INTRODUCTION

Unwanted immune reactions in patients with autoimmune diseases or recipients of allogeneic organ transplants are routinely suppressed by immunosuppressive agents. Drugs such as corticosteroids, azathioprine, cyclosporine A, and antilymphocyte globulin (ALG) which are used to control rejection, graft-versus-host disease (GVHD), or autoimmunity, have broadly suppressive effects on cell-mediated immune function. Consequently, treated patients are susceptible to infections in general, and to viral infections in particular.

Although the immunosuppressive capacity of ALG is well established, the efficiency of different ALG preparations in clinical transplantation is variable. This might be due to the composition of ALG with respect to antigen specificity, (sub) class distribution, and species in which it has been raised (Capel, van Rijs & Koene, 1983). The advances of hybridoma technology have rekindled hopes for the use of monoclonal antibodies (MoAb) as immunosuppressive agents, thus eliminating the problem of great variability in ALG preparations.

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Several reports have focussed their attention on the use of MoAb, both in animal models (Jonker, Goldstein & Balner, 1983; Michaelides, Hogarth & McKenzie, 1981; Nakayama & Uenaka, 1985) and clinical transplantation (Gratama *et al.*, 1984; Martin *et al.*, 1984; Remlinger, Martin & Hansen, 1984). However, the use of MoAb directed against T cells, T cell subsets, or Ia determinants as potent immunosuppressive agents does not solve the problem of side effects caused by general immunosuppression.

Our concept of immunosuppression is based on the inhibition or elimination of the specific antigen-activated lymphocytes without the induction of changes in the pool of resting lymphocytes. MoAb against surface antigens with their expression restricted to activated lymphocytes may be useful in this respect. Interleukin 2 (IL-2) receptors have been detected on the surface of antigen (or mitogen)-activated lymphocytes, but not on resting cells (Diamantstein & Osawa, 1984; Osawa & Diamantstein, 1984); so MoAb specific for the IL-2 receptor can fulfill the conditions for a more specific immunosuppression.

In this report, we show that the acute graft-versus-host reaction (GVHR) across a strong MHC barrier in mice can be suppressed by treatment with AMT-13, an MoAb directed against the IL-2 receptor on activated mouse lymphocytes (Osawa & Diamantstein, 1984). The therapeutical capacity of the AMT-13 MoAb is compared with the effects of treatment with L3T4, an MoAb which recognizes the T helper subset.

MATERIALS AND METHODS

Mice. Males of the inbred strains DBA/2, 6–12 weeks old, and (DBA/2 × C57BL/6) F1 male hybrids, 4–8 weeks old, were used.

Cells. Spleen and lymph node cell populations were prepared as single cell suspensions. The cells were washed three times and resuspended in Dulbecco's solution (PBS).

GVHR-assay. For the local GVHR-assay the popliteal lymph node (PLN)-assay was used (Ford, 1978). GVHR was induced by injecting young (DBA/2 × C57BL/6) F1 mice with 3×10^6/50 μl DBA/2 strain spleen and lymph node cells (ratio 1:1) into the foot-pad. One foot per recipient was injected. The control group was injected with syngeneic F1 cells.

Seven days after injection the recipients were killed, their PLN of both sides were excised and cleaned of adherent fatty tissue. The nodes were then weighed. The GVHR-index was determined by the following formula: GVHR-index = weight of the ipsilateral PLN/weight of the contralateral PLN.

Monoclonal antibodies. The preparation and specificity of the MoAb AMT-13 (IgG 2a) are described elsewhere (Osawa & Diamantstein, 1984). The MoAb L3T4 (ATCC/TIB 207, clone GK 1.5.) is a rat MoAb (IgG 2b) which recognizes a T cell surface antigen that is expressed by the helper/inducer subset of murine T cells. The mice were treated with 1 mg purified MoAb/kg body weight/day, intraperitoneally, on days 0, 1, 3, and 5 after cell transfer. A control group received a treatment with an unrelated MoAb of the appropriated subclass. For the immunofluorescence assay the MoAb anti-Lyt 2 and antitheta (obtained from Becton-Dickinson) were additionally used. As second antibody a biotinylated goat anti-rat Ig antibody and FITC-labelled avidine (obtained from Becton-Dickinson) were used.

Immunofluorescence. The mesenteric lymph nodes and the GVHR-stimulated PLN of five animals in each group were removed and single cell suspensions were prepared. The heparinized peripheral blood was diluted with PBS and the lymphocytes were separated by density gradient centrifugation and then washed three times with PBS. Cells (10^6) were pelleted in each tube and incubated with the appropriate MoAb (10 μg/ml) for 30 min on ice. After washing, the cells were incubated with the biotinylated goat anti-rat Ig antibody (diluted in PBS containing 2% mouse normal serum) for 30 min. Then, the cells were washed and avidine-FITC was added to them. The cells were studied under a fluorescence microscope (Zeiss-Ikon). At least 200 cells were counted each time. All washing procedures and dilutions were carried out in PBS containing 0·1% NaN3 and 2% fetal calf serum.

RESULTS

Determination of the optimal cell dose for the local GVHR. The dose/response plot after injection of graded doses of parental strain lymphocytes into the foot-pad of F1 hybrid mice were recorded. The linear range was from $0{\cdot}5 \times 10^6$ to 5×10^6 donor cells in the used strain combination. At 5×10^6 cells there was a small effect on the contralateral node (data not shown), so we used a dose of 3×10^6 donor cells for the following experiments.

Effects of in-vivo administration of L3T4. Figure 1 shows the results of the in-vivo administration of the MoAb L3T4 that recognizes the T helper subpopulation. The local GVHR was induced on day 0 by injecting F1 mice with parental strain lymphocytes. The mice were given injections of L3T4 (1 mg/kg/day) on days 0. 1, 3, and 5. After day 7, the GVHR-index was determined.

The treatment of F1 mice with L3T4 significantly reduced ($P < 0{\cdot}05$) the GVHR-stimulated lymph node enlargement in all treated animals (about 50% inhibition). Alternatively, the treatment with a control MoAb had no influence on the GVHR-index. We also studied the influences of L3T4 treatment on the distribution of T lymphocyte subsets in the peripheral blood and in both control (mesenteric) and GVHR-stimulated (PLN) lymph nodes. As shown in Table 1, the L3T4 treatment caused a dramatic decrease in the proportion of $L3T4^+$ cells in the circulation and a marginal reduction in the numbers of these cells in the lymph nodes. In addition, the absolute number of circulating lymphocytes in the blood of the treated group dropped by an average of 50% ($8{\cdot}1 \times 10^9$/l vs $4{\cdot}4 \times 10^9$/l).

AMT-13 cells represent a minor population (< 1%) in control lymph nodes and in peripheral blood. In the GVHR-stimulated lymph nodes, the frequency of $AMT\text{-}13^+$ cells was increased to *c.* 10%. In the L3T4-treated group the proportion of $AMT\text{-}13^+$ cells in the GVHR-lymph nodes was reduced to the normal level (Table 1).

Effects of in-vivo administration of AMT-13. As shown in Fig. 1, the AMT-13 treatment of F1 mice was sufficient to inhibit the local GVHR. The dosage and method of application were the same

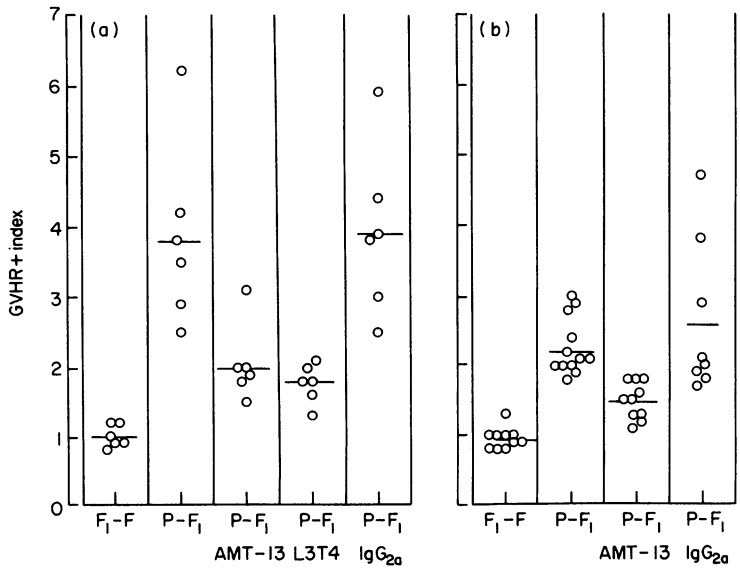

Fig. 1. Effects of treatment with the MoAb L3T4 (T helper cells) and AMT-13 (IL-2 receptor) on the local graft-versus-host reaction in mice. Local GVHR was induced as described in Materials and Methods. The animals were treated with either L3T4 or AMT-13 i.p. on days 0, 1, 3, and 5. After day 7, the GVHR-index was determined. (a) Experiment 1. (b) Experiment 2.

Table 1. Distribution of T lymphocyte subsets in lymph nodes and peripheral blood after treatment with L3T4 or AMT-13

Group No./treatment	% positive cells				
	theta	L3T4	Lyt 2	L3T4/Lyt2	AMT-13
1 control					
blood	73	48	25	1·9	0–1
normal LN*	65	45	21	2·1	1
GVHR-LN†	70	57	29	2·0	10
2 AMT-13					
blood	70	45	26	1·7	0–1
normal LN	63	43	22	2·0	0
GVHR-LN	70	*45*	26	1·7	*3*
3 L3T4					
blood	*49*	*20*	32	*0·6*	0–1
normal LN	61	*38*	29	*1·3*	0
GVHR-LN	67	*41*	28	*1·5*	*2*

* Mesenteric lymph node.

† GVHR-stimulated popliteal lymph node.

Local GVHR was induced by injecting F1 mice with parental lymphocytes. The animals (five in each group) were treated with the MoAb on days 0, 1, 3, and 5. After 7 days the lymphocytes of the various lymphoid compartments were prepared and stained with a set of MoAb. Standard errors of the mean percentages were less than 5% and are not shown in the Table. The italicised numbers indicate significant changes in the T cell subset pool by treatment with the MoAb.

as with L3T4 (see above). The small differences in the suppression of the GVHR between the L3T4-treated group and the AMT-13-treated group was not significant ($P > 0{\cdot}05$).

In both the peripheral blood and control lymph nodes, the AMT-13 treatment had no influence on the distribution of the T cell subsets (Table 1). Only in the GVHR-stimulated PLN the proportion of T cells with the helper/inducer phenotype and the ratio between the two major T cell subsets were decreased. Furthermore, the increase in the proportion of AMT-13$^+$ cells which was observed in these lymph nodes was brought down by treatment with AMT-13.

DISCUSSION

When parental strain lymphocytes are injected into F1 hybrid (i.e. semi-allogenic) animals, the recipients are genetically tolerant of parental strain cells but the grafted T lymphocytes on the other hand react against allogeneic histocompatibility antigens of the host and thus induce an acute GVHD (Gleichmann *et al.*, 1984), which in the early phase is characterized by an activation of MHC class II-restricted donor T cells which in turn results in a transient lympoproliferation and B cell activation during the first week of GVHD followed by a phase of allosuppression (Gleichmann *et al.*, 1984). We measured the lymphoproliferation of the first phase by using the popliteal lymph node assay.

It is known that the first phase of GVHR is associated with a proliferation of the donor T helper compartment (Gleichmann *et al.*, 1984). According to the interleukin 2 (IL-2) concept, proliferation of T cells in response to antigens depends on expression of IL-2 receptors at the surface of antigen-activated T cells and on the binding of IL-2 to the receptor. We therefore, tested in the local GVHR-model, the effficiency of a treatment with the MoAb AMT-13 specific for the IL-2 receptor on mouse lymphocytes, in relation to the treatment with the MoAb against the T helper/inducer subset (L3T4).

Several groups have reported that MoAb against T cells (Gratama *et al.*, 1984; Remlinger *et al.*, 1984) or T cell subsets (Jonker *et al.*, 1983; Michaelides *et al.*, 1981; Nakayama & Uenaka, 1985) could be powerfully immunosuppressive in autoimmune and transplantation models.

In this paper, we have shown that in agreement with these results the local GVHR could be significantly inhibited by treatment with an MoAb against the T helper subset (L3T4).

The administration of MoAb against T cells or T cell subsets causes a decrease in the appropriate lymphocyte population, as demonstrated in this study (Table 1) and in studies done by others on animal models (Jonker *et al.*, 1983; Michaelides *et al.*, 1981) and human patients (Gratama *et al.*, 1984; Martin *et al.*, 1984; Remlinger, *et al.*, 1984). Such general immunosuppressive treatment can be associated with a substantial risk of infections especially by viruses.

As shown in Fig. 1, the suppression of the local GVHR by treatment with AMT-13 was comparable with the effects of L3T4 administration. Evidently, the treatment with the MoAb against the IL-2 receptor (AMT-13) caused no changes in the distribution of T lymphocyte subpopulations in both peripheral blood and control lymph nodes. However, in the GVHR-stimulated lymph nodes the lymphoproliferation was associated with an increase of IL-2 receptor-bearing lymphocytes. The treatment with the MoAb L3T4 and AMT-13 was followed by a decrease in the numbers and proportions of both IL-2 receptor-bearing lymphocytes (AMT-13$^+$) and T helper cells (L3T4$^+$) in these stimulated lymph nodes (Table 1), which suggest that these IL-2 receptor-bearing cells belonged mainly to the L3T4$^+$ subset. Obviously, only the small subset of IL-2 receptor-bearing lymphocytes was influenced by the treatment with AMT-13.

One mechanism of action of AMT-13 could be the direct blocking of IL-2 binding and IL-2-dependent proliferation as has been demonstrated *in vitro* (Diamantstein & Osawa, 1984; Osawa & Diamantstein, 1984). However, the concentration of MoAb which is necessary for inhibition of IL-2-dependent proliferation *in vitro* has to be very high, because the affinity of IL-2 to the receptor is higher (Osawa & Diamantstein, 1984). The most likely mechanism of the in-vivo effect of the MoAb against the IL-2 receptor is opsonization of the receptor-bearing cells by antibody followed by removal of coated cells by the reticuloendothelial system, rather than complement-dependent cell killing (Capel *et al.*, 1983; Jonker *et al.*, 1983).

In contrast to the local GVHR, AMT-13 treatment had only a marginal influence on the systemic GVHR as judged by the spleen weight assay (Ford, 1978). In the both neonatal and adult irradiation model the AMT-13 injections did not inhibit the spleen enlargement significantly (data not shown). The reason for the discrepancy between systemic and local GVHR is not yet clear.

Very recently, we found that MoAb against the IL-2 receptor effectively suppress the development of acute autoimmune encephalomyelitis (Wekerle & Diamantstein, 1986) and of adjuvant arthritis (Smith *et al.*, 1986) induced in naive recipients by transfer of specific T lymphocytes, or allograft rejections (Kupiec-Weglinski *et al.*, 1986). Here, we have proved the effectiveness of such treatment in a different model by showing the suppression of local GVHR in mice.

MoAb against antigens like the IL-2 receptor, the expression of which is restricted to activated lymphocytes, might become useful for a more specific immunosuppression with limited side effects.

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